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THIS IS A SUMMARY OF THE PROJECT WORK PLAN DEVELOPED TO STUDY NATURAL AND ENHANCED MICROBIAL DEGRADATION OF C6H6, MEC6H5, AND XYLEN IN AQUIFER CORES FROM THE SOUTH TANK FARM PLUME. LABORATORY SCREENING EXPERIMENTS WILL BE PERFORMED IN SOIL-GROUND WATER MICROCOSMS SUPPLEMENTED WITH OXYGEN AND/OR NUTRIENTS. THE TESTS ARE SCHEDULED TO BEGIN IN THE FOURTH QUARTER OF 1990 AND CONTINUE INTO 1991.

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PROJECT STATUS REPORT

LABORATORY STUDIES ON BIODEGRADATION OF ORGANICS IN SOUTH TANK FARM PLUME AQUIFER SAMPLES

Biodegradation of Organics in STFP
J. P. Salanitro, H. L. Wisniewski

Summary: Bioremediation of organics in nutrient-amended groundwater has been successful for aromatic hydrocarbons like benzene, toluene, and xylenes. These compounds are present in the RMA South Tank Farm Plume (STFP) including dicyclopentadiene and bicycloheptadiene. A project work plan has been developed to study natural and enhanced microbial degradation of these compounds in aquifer cores from the STFP. Laboratory screening experiments will be performed in soil-groundwater microcosms supplemented with oxygen and/or nutrients.

Background: An examination of the literature on the biodegradation of aromatic hydrocarbons such as benzene, toluene, xylenes, and chlorobenzene indicates that these are readily metabolized by a wide variety of naturally-occurring bacteria and fungi in soils (Gibson, 1984). Initial enzymatic attack on the ring is through cis-diol formation (oxygenases), followed by ring opening and oxidation to a dicarboxylic acid derivative. This acid is further metabolized to CO through one or more metabolic pathways (e.g., citric acid cycle) in the cell. Half-life values for the degradation of these hydrocarbons in soil, groundwater, or bacterial cultures varies from 0.5-15%/day at BTX concentrations of 0.01-50 ppm in laboratory studies (Table 1). These rates are somewhat higher than those obtained from field estimations (about 1%/day) where natural unassisted degradation occurs. This suggests that soils may have a large capacity to

metabolize high levels of aromatic hydrocarbons and factors such as aeration, nutrients, and diverse soil microbe populations may be important in stimulating rapid and extensive depletions.

No data exists in the literature on the biodegradation of the cycloalkenes, dicyclopentadiene (DCPD) and bicycloheptadiene (BCHPD), in soils or cultures. These compounds may be biotransformed like the drins-type hydrocarbons, dieldrin and endrin, because of their similarity in structure. Enzymatic attack would be through the formation of epoxide, alcohol, ketone, or carboxylic acid derivatives. The potential for enhanced degradation of these cycloalkenes needs to be assessed in laboratory soil screening tests with nutrient and inoculum amendments. Chlorobenzene is most likely degraded similarly to benzene and higher biotransformation rates (6%/day) with 20 and 660 ppm have been observed with cultures of soil and sewage bacteria. These are also higher than those observed in unstimulated soils (0.4 and 1.6%/day).

Project Description for Laboratory Screening Program: The basic features of a microbiological and analytical experimental protocol to determine the potential for bioremediation of aromatic hydrocarbons, cycloalkenes (DCPD, BCHPD), chlorobenzene and chloroform in groundwater at initial levels of 100 ppb-50 ppm through nutrient amendments are:

1) Devise appropriate lab test systems to assess hydrocarbon degradation. (See Figure 1.)

2) Verify analytical methods (extraction techniques and gas chromatography) for recovery and identification of hydrocarbons from groundwater.

3) Determine upper concentration limits (threshold) for degradation and/or inhibition.

4) Determine degradation rates by natural groundwater microbial populations in the presence of O_2 (or H_2O_2) and nutrient (N, P, Fe) amendments.

5) Determine total numbers of viable aerobic and anaerobic bacteria in STFP cores.

Project Timing: These experiments are scheduled to begin in the fourth quarter, 1990 and continue into 1991. Preliminary results should be available by second quarter 1991 depending on how rapidly the degradation occurs.

TABLE 1

SUMMARY OF BIODEGRADATION DATA

Compound	Concentration tested, ppm	Test System[a)]	Degradation %/Day	Ref.
Benzene	1.8-2.4	Soil/GW (field)	0.07-1.1	Barker & Patrick (1985)
Toluene	1.8-2.6	Soil/GW (field)	1.4	Barker & Patrick (1985)
	0.01-5	Soil/GW	7-33	Internal Shell Studies
Xylenes	0.05-5	Soil/GW	2.5-71	Mahadevaiah & Miller (1986)
	.01-5	Soil/GW	7-50	Kuhn et al. (1985)
	20	Soil (denitrifying)	16	Zeyer et al. (1986)
BTX	1-56	Various cultures	12.5	Jamison et al. (1976)
Chlorobenzene	1	Soil	1.6	Wilson (1981)
	20	Soil	0.4	Haider et al. (1981)
	660	Soil/sewage isolate	14	Reineke & Knackmuss (1984)
	20	Nocardia/ Pseudomonas	6-10	Haider et al. (1981)
Dicyclopentadiene (DCPD)	-[b)]	-	-	-
Bicycloheptadiene (BCH)	-	-	-	-

References

Barker, J. F. and G. C. Patrick, 1985. Natural attenuation of aromatic hydrocarbons in a shallow sand aquifer. Proc. NWWA/API Conf. on Petroleum Hydrocarbons and Organic Chemicals in Groundwater (November).

Gibson, D. T. (Ed.), 1984. Microbial degradation of organic compounds. Marcel Dekker, NY.

Haider, K., G. Jagnow, R. Kohnen, and S. U. Lim, 1981. Degradation of chlorinated benzenes, phenols and cyclohexane derivatives by benzene- and Phenol- utilizing soil bacteria under aerobic conditions. In Decomposition of toxic and non-toxic organic compounds in soil, M.R. Overcash (Ed.). Ann Arbor Science, p. 207-223.

Jamison, V., C. G. Enfield, W. J. Dunlap, R. L. Cosby, D. A. Foster, and L. B. Baskin, 1981. Transport and fate of selected organic pollutants in a sandy soil. In Biodegradation of high-octane gasoline. Proc. 3rd Intl. Biodegradation Symp., Appl. Sci. Publ., Ltd. London, pp. 187-196.

Kuhn, E.P. and R. P. Schwarzenbach, 1986. Rapid microbial mineralization of toluene and 1.3-dimethylbenzene in the absence of molecular oxygen. Environ. Sci. Technol 19:961-968.

Mahadevaiah, B. and G. D. Miller, 1986. Application of microcosm technology to study the biodegradation potential of a subsurface alluvial material exposed to selected petroleum hydrocarbons. Sixth Natl. Symp. and Expos. Aquifer Restor and Groundwater Monitor (May).

Reineke, W. and H. J. Knackmuss, 1984. Microbial metabolism of haloaromatics: isolation and properties of a chlorobenzene degrading bacterium. Appl. Environ. Microbiol 47:395-402.

Wilson, J. T. Microbial metabolism of haloaromatics: isolation and properties of a chlorobenzene-degrading bacterium. J. Environ. Qual. 10:501-506.

Zeyer, T. E. P. Kuhn, R. P. Schwarzenbach, 1986. Rapid microbial mineralization of toluene and 1,3-dimethylbenzene in the absence of molecular oxygen. Appl. Environ. Microbial. 52:944-947.

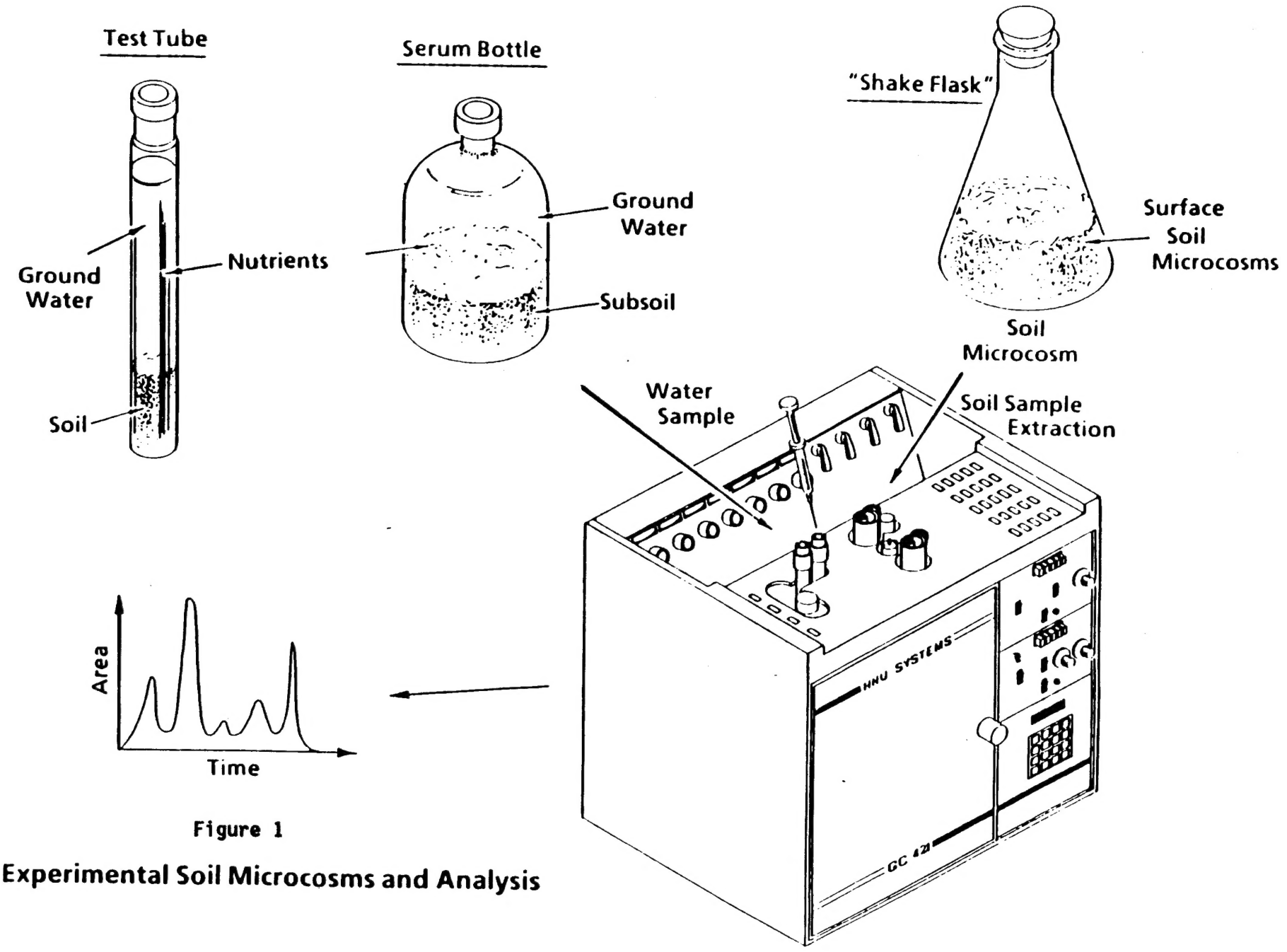

Figure 1

Experimental Soil Microcosms and Analysis